

Remarks

Claims 1-2, 4, and 6-7 stand rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Nehls. It is respectfully submitted that this rejection has been rendered moot by the amendment of claim 1 herewith. Claim 1 has been amended to require that the yeast targeting cassette (YTC) does not include a yeast selectable marker. Nehls discloses and his method requires that his purported counterpart of the YTC (what Nehls calls a “positive selection cassette vector”) includes a yeast selectable marker. By developing a method that avoids the need for this yeast selectable marker, Applicants have created a superior system.

The Applicants’ system results in a final product, the gene vector, which when used to create recombinant mice and the like, does not bring into the mammalian cell this extra yeast selectable marker element. The yeast selectable marker of Nehls is an entire coding sequence with yeast promoter elements and the like, and these promoter elements might allow some low level of transcription of the yeast selectable marker gene product within the genetically modified mammal. It is possible that this low level of yeast gene product within the mammal will impact the traits and/or survival of the mammal. Thus, when someone wishes to investigate the effect of the targeted genetic modification on a mammal or a mammalian cell, this effect will be further complicated by the Nehls system, as compared to the use of Applicant’s inventive method. Thus, when using Applicants’ method, it is more likely that all observed changes of the genetically modified mammal are due to the targeted mutation, and NOT due to the presence of yeast proteins in the cells. It is respectfully requested that the rejection of claims 1-2, 4, and 6-7 be withdrawn in view of Applicant’s amendment and arguments.

Claim 3 stands rejected under 35 U.S.C. §103(a) as allegedly being obvious over Nehls in view of Lewin. Claim 5 stands rejected under 35 U.S.C. §103(a) as allegedly being obvious over Nehls in view of Brocard. Claim 8 stands rejected under 35 U.S.C. §103(a) as allegedly being obvious over Nehls in view of Luo. It is respectfully submitted that these rejections have been rendered moot by the amendment of claim 1 herewith. Thus, it is respectfully requested that the rejections of claims 3, 5, and 8 be withdrawn in view of Applicant’s amendment and arguments.

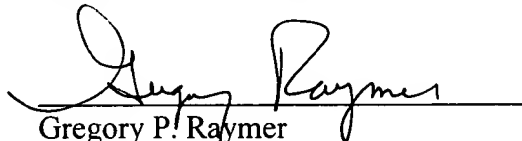
The Commissioner is hereby authorized to charge any additional fees required, or to credit any overpayment, to Deposit Account No. 16-1445.

A Notice of Allowance is courteously solicited.

Respectfully submitted,

Date:

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pCosRKODelta plasmid DNA was mixed with pCosRKO plasmid DNA in molar ratios of 1:1, 1:10, 1:100, and 1:1000. For each yeast transfection 100 ng of DNA from one of the above mixtures was used. To prepare a DNA mixutre for transfection, DNA was linearized with the enzyme I-CeuI that cuts between two 50
 5 base pair repeats in order to enhance transfection efficiency as described by Raymond et al. (BioTechniques, 27:892-96 (1999)). The DGY63 yeast strain (R.D. Geitz, University of Manitoba) was transfected with 100 ng of I-CeuI digested pCosRKO and either 160 ng of the hsvTK-YTC described above according to the TRAFO method described (Agatep et al., Technical Tips Online,
 10 (~~http://tto.trends.com~~) (1998)) with the exception that all solutions and media were made with ES Cell Qualified Ultra Pure H₂O (Specialty Media, Phillipsburg, NJ; cat # TMS-006-A). From the final suspension of transformed yeast in this protocol, 0.5 µl is diluted to 100 µl with sterile water and plated onto a 100 mm SC-Trp plate (Agatep et al., Technical Tips Online, (~~http://tto.trends.com~~) (1998)). In addition 5µl
 15 , 50 µl , and 500 µl were diluted to 3 ml and plated onto 500 cm² SC-Trp plates (Agatep et al., Technical Tips Online, (~~http://tto.trends.com~~) (1998)). The 1.0 µl aliquots were used to deduce the yeast transformation efficiency per µg of DNA. From this efficiency the number of colonies on each of the 500 cm² plates was estimated. The yeast colonies were scraped off of each plate into 1-10 ml of YAPD
 20 media, spun down by centrifugation, and plasmid DNA prepared using Zymoprep™ (Zymo Research, Orange, CA). From the approximately 50 µl solution of DNA that was prepared, 3 µl was used to electroporate GeneHogs® electrocompetent bacterial cells (Research Genetics, Huntsville, AL). The electroporated bacteria were plated onto LB plates containing kanamycin (5 µg/ml). The number of
 25 kanamycin resistant clones was recored. Table 1 shows the results of this experiment.

Table 1

Condition	Yeast cfu/plate	KAN cfu/plate	Transformation Efficiency
1:1	163	lawn	3.26 X10 ⁶
	1630	77	
	16,300	66	
	163,000	14	

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